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THE PHYSICAL EFFECT OF ANESTHETICS UPON LIVING PROTOPLASM.[1]

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Claude Bernard and many others after him have pointed out that anesthesia is a phenomenon common to all animals and plants. Vital processes of the most diverse types can be checked without destroying life itself.

Because of the general nature of anesthesia and because in many cases the same substances in not very different concentrations anesthetize widely different forms it has usually been felt that anesthetics act primarily upon some property common to all life. Many have believed that anesthetics directly lower oxygen consumption. But even the consumption of oxygen is not a sufficiently widespread process for there are some organisms which do not require any oxygen and these can be anesthetized.[2] Recently several prominent workers have urged the view that anesthetics have an effect on the permeability of the plasma membrane. It is claimed that they decrease permeability or at least prevent the increase of permeability which is supposed to follow stimulation. R. S. Lillie[3] has summarized the literature on anesthesia and in his excellent review he has brought together most of the evidence in favor of the permeability theory. Some of the opposing evidence is cited by Traube.[4] There are numerous other theories of anesthesia.

Many have tried to associate the phenomenon of anesthesia with what is no doubt one of the most fundamental properties of protoplasm, its viscosity. From the very first description of

[1] Contribution from the Zoölogical Laboratory, University of Michigan.

[2] Vészi, J., *Arch. ges. Physiol.*, 1918, CLXX., 313.

[3] Lillie, R. S., BIOL. BULL., 1916, XXX., 311.

[4] Traube, J., *Arch. ges. Physiol.*, 1919, CLXXVI., 70.

protoplasm, it has been known that the living substance is viscous. Like all other viscous colloidal substances, no doubt protoplasm can undergo sudden marked increases or decreases in viscosity. These changes in viscosity are among the most outstanding characteristics of those chemical substances which enter into the composition of protoplasm. It is to be expected therefore that viscosity changes play a large part in the mechanics of vital processes.

In colloidal solutions viscosity changes may be of two sorts. Oftentimes the change is only slight but in other cases there is so marked an increase in viscosity that the colloidal solution loses many of the properties of a liquid and is apparently a solid. Of course it is not truly a solid, for the solid state is properly associated with crystal form. Highly viscous colloidal liquids are known as semi-solids or gels. There is no sharp boundary line between such gels and ordinary liquid colloidal solutions or sols. Every intergradation exists. Colloidal solutions when treated with reagents sometimes undergo gel formation but in other cases there is a precipitation. In the latter instance there is often a transitory increase in the viscosity of the liquid, accompanied perhaps by the formation of an unstable gel, then as the colloidal substance passes out of suspension, the viscosity of the liquid decreases again. In a test-tube there is apparently a sharp difference between gelation and precipitation, but this would not be true within a cell. The entire cell is often smaller than a single flake of the precipitate.

From the first the importance of protoplasmic viscosity changes has been clearly recognized by biologists. Many theories of diverse vital processes have been based on the idea of a change in viscosity or a change in state of aggregation. Claude Bernard himself believed that anesthesia was due to a "semi-coagulation" of the protoplasm.[1] Since Claude Bernard many have held a similar view.

In order to support this view, some authors have endeavored to show a physical effect of anesthetics on various colloidal solutions. Höber and Gordon[2] showed that chloroform vapor and

[1] Bernard, C., Leçons sur les anesthésiques et sur l'asphyxie, Paris, 1875.

[2] Höber, R., and Gordon, Dora, *Beitr. Chem. Physiol. u. Path.*, 1904, V., 432.

ether vapor tend to prevent the precipitation of a 0.4 per cent. lecithin suspension by barium or calcium chloride. Moore and Roaf[1] found that various anesthetics in high concentration caused a precipitation of the proteins of blood serum. Koch and McLean[2] maintained that anesthetics have no consistent effect on the state of aggregation of lecithin suspensions. Warburg and Wiesel[3] studied series of urethanes, alcohols, nitriles and lactones. They found in every case that the higher members of the series had a more pronounced precipitating action on yeast extracts. The precipitating power was correlated with the power to check fermentation. Battelli and Stern[4] state that all anesthetics possess the power of precipitating nucleoproteins from their watery extracts. Schryver[5] found that the time of gel formation of sodium cholate was lengthened by the presence of anesthetics. Thomas[6] found that anesthetics caused well-marked changes in the viscosity of lecithin suspensions. Generally in higher concentration an increase in viscosity occurred.

With the exception of Koch and McLean all of the above authors believe that the physical effects produced by anesthetics upon colloidal solutions are of importance to the theory of anesthesia. But whereas some hold that the effect primarily concerns the viscosity of the substances in the plasma membrane, others apparently believe that the colloids of the cell interior are involved. Not only is there disagreement as to where the effect is produced but there is a decided difference of opinion as to what sort of a change occurs. Some find that anesthetics cause a decrease in viscosity, others claim the reverse. This divergence in results is in part explained by the fact that different authors have used widely different concentrations of anesthetics. Moreover the experiments have been performed upon various types of colloidal solutions.

Although the results on inanimate substances are interesting, it is obviously more significant to measure the viscosity of the living

[1] Moore, B., and Roaf, H. E., *Proc. Roy. Soc.*, 1906, B. LXXVII., 86.
[2] Koch, W., and McLean, F. C., *Journ. Pharm. Exp. Therap.*, 1910, II., 249.
[3] Warburg, O., and Wiesel, R., *Arch. ges. Physiol.*, 1912, CXLIV., 465.
[4] Battelli, F., and Stern, L., *Biochem. Zeit.*, 1913, LII., 226.
[5] Schryver, S. B., *Proc. Roy. Soc.*, 1916, B. LXXXIX., 176.
[6] Thomas, A., *J. Biol. Chem.*, 1915, XXIII., 359.

protoplasm itself. This can very simply be done by the centrifuge method. In a recent paper on cell division[1] I have recorded a number of measurements of the viscosity of the anesthetized protoplasm of sea-urchin eggs. The method of measurement is described there as well as in a previous paper.[2] These results give the effect on protoplasmic viscosity of various anesthetics at the very concentration at which they are effective in producing anesthesia.

One fault that might perhaps be found with some of the prevailing theories of anesthesia is that they are based for the most part on very general evidence. It is only rarely that any one process has been thoroughly studied. And yet in order to understand anesthesia in general it is perhaps essential first to concentrate attention on some one process, preferably a simple one. An explanation of this single case can then perhaps be applied to other cases.

I have studied particularly the process of cell division in sea-urchin eggs. Anesthesia in sea-urchin eggs was first studied by Fühner[3] who interpreted his results as favoring the Overton-Meyer theory. R. S. Lillie[4] later determined the effective concentration for many anesthetics. He also attempted to show that anesthetics decrease the permeability of the plasma-membrane.[5]

In the process of cell division in the sea-urchin egg I have shown that there are certain marked viscosity changes.[6] These are always present. First there is a sharp increase in viscosity and this is followed by a decrease.

What then is the effect of anesthetics on the cytoplasmic viscosity of these eggs? In seeking to answer this question we must study every substance which prevents cell division without killing the egg. Even those substances which produce injurious effects must be considered. According to some writers these are not true anesthetics, but such a distinction is not a wise one. Practically every anesthetic produces some injury if the exposure is

[1] Heilbrunn, L. V., *J. Exp. Zoöl.*, 1920, XXX., 211.
[2] Heilbrunn, L. V., Biol. Bull., 1915, XXIX., 149.
[3] Fühner, H. —., *Arch. Exp. Path. u. Pharmacol.*, 1904, LII., 69.
[4] Lillie, R. S., *J. Biol. Chem.*, 1914, XVII., 121.
[5] Lillie, R. S., *Amer. J. Physiol.*, 1918, XLV., 406.
[6] Heilbrunn, L. V., *J. Exp. Zoöl.*, 1920, XXX., 211.

long enough. After a few hours ether is decidedly harmful to higher animals, and yet no one would question its claim to being an anesthetic. Other anesthetics produce more marked injuries. There is every gradation between those which produce slight injury and those which cause considerable damage. Where then shall we draw the line? I propose to consider as an anesthetic any substance which stops a vital process without killing the cells in which the process occurred. We can then distinguish between the less injurious and the more injurious anesthetics.

In the following table all the substances listed produce anesthesia in the sea-urchin egg. The concentrations represent per cents. in sea-water. At the concentration indicated in the first

	Anesthetic concentration	Lethal concentration
Ether	2 %	4 %
Chloroform	0.13%	1 % (emulsion)
Chloral hydrate	0.25%	1 %
Nitromethane	2 %	3 %
Paraldehyde	4 %	8 %
Acetone	5 %	10 %
Ethyl nitrate	0.4 %	
Ethyl acetate	2 %	5 %
Ethyl butyrate	0.25%	0.5%
Acetonitrile	4 %	5 %
Propyl alcohol (normal)	1 %	
Amyl alcohol	0.6 %	1 %
Phenyl urethane	4/5 saturated	saturated ($=<0.5\%$)
Ethyl urethane	1.5 %	3 %

column, in every case a marked lowering of the viscosity of the egg protoplasm could be demonstrated. This was proven by centrifuge tests. Normal eggs in sea-water and anesthetized eggs were centrifuged simultaneously, the normal eggs being placed in one tube, the anesthetized eggs in another. At a speed which produced no movement of granules in the cytoplasm of the normal eggs, the granules in the cytoplasm of the anesthetized eggs were thrown completely into one half of the egg. The difference was in every case very striking. The normal eggs appeared evenly opaque. The anesthetized eggs had half or more of their cytoplasm perfectly transparent. The viscosity of the anesthetized cytoplasm was undoubtedly many times less than that of the normal eggs.

The concentration of anesthetic which produces a marked lowering of protoplasmic viscosity is the exact concentration which prevents cell division. Lower concentrations produce less decided effects on cytoplasmic viscosity and in these cell division is more apt to occur. As the concentration of the anesthetic is increased, the decrease in cytoplasmic viscosity becomes more and more pronounced until suddenly a turning point is reached. At this point gelation or coagulation occurs. Eggs subjected to such high concentrations of anesthetic undergo a decided increase in cytoplasmic viscosity. When these eggs are centrifuged at high speed the cytoplasmic granules remain fixed and do not move. Compared with normal eggs the protoplasm of these coagulated eggs is much more viscous. The concentrations noted in the second column of the table are those which were found to produce a sharp increase in protoplasmic viscosity. Such an increase in viscosity is in general irreversible. In practically every case the eggs did not recover.

My results with ether agree fairly well with the older observations of Heilbronn[1] on plant protoplasm. Heilbronn's measurements were made by timing the drop of starch granules. He found that dilute ether solutions caused a decrease in protoplasmic viscosity; more concentrated solutions caused increased viscosity. Heilbronn was inclined to associate anesthesia with the more concentrated solutions, which caused a stiffening in the protoplasm, a "Plasmastarre." Perhaps this is true for plant cells, but it is rather difficult to decide when plant cells are anesthetized. Heilbronn found that the "Plasmastarre" was in general reversible.

In medicine cold is a well-known anesthetic. Low temperatures also anesthetize sea-urchin eggs. With decreasing temperatures the protoplasm becomes less and less viscous. Even at 10° C. there is a noticeable difference as compared to room temperature. At —3° C. centrifuge tests both with fertilized and unfertilized eggs showed a decided lowering of viscosity. In such eggs cell division is prevented, although the eggs remain uninjured. Temperatures somewhat lower than —3° produce an increase rather than a decrease in viscosity. If the sea-water

[1] Heilbronn, A., *Jahrb. wiss. Bot.*, 1914, LIV., 357.

in which the eggs are contained is allowed to freeze, the egg cytoplasm becomes coagulated. Such eggs are permanently injured by the treatment. In one experiment eggs were exposed to a temperature of —6° C. for 10 minutes. In spite of the fact that no ice was observed in the tube containing the eggs, the protoplasm when tested was found to be coagulated. Thus in the case of cold also there is apparently a turning point, at which the viscosity of the cytoplasm no longer decreases but suddenly shows a marked increase.

When sea-water is diluted, the resultant hypotonic solution is one of the best (*i.e.*, least injurious) anesthetics. A solution made up of equal parts of sea-water and distilled water is an excellent anesthetic. There is nothing unusual about this, for is has long been known that water anesthetizes nerves. This knowledge dates back as far as 1869,[1] and water has occasionally been used in various surgical operations. In the sea-urchin egg those dilutions of sea-water which prevent cell division are the very ones which produce a marked decrease in cytoplasmic viscosity. On the other hand, too great a dilution causes coagulation; this effect is produced when the eggs are dropped into distilled water.[2]

Not all anesthetics cause a decrease in cytoplasmic viscosity. Some produce quite the opposite effect.

Many students of anesthesia confine their attention to fat solvent anesthetics. This in spite of the fact that magnesium sulphate in concentrated solution is apparently the best anesthetic for most marine invertebrates. As far as I can discover magnesium sulphate was first used by Tullberg.[3] Its effect appears to be largely an osmotic one, for the salt acts best in hypertonic solution. This is also indicated by the observations of Loeb[4] on hydroids. Loeb found that the regeneration of hydroids could

[1] Braun, H., Local Anesthesia, English translation by Shields, Philadelphia, 1914, p. 62.

[2] Heilbrunn, L. V., BIOL. BULL., 1915, XXIX., 149.

[3] Tullberg, T., *Verhandl. d. biolog. Vereins Stockholm*, 1891, IV., Nr. 1–2, p. 4, cited after Gerould, J. H., *Bull. Muss. Comp. Zoöl.*, Harvard, 1896, XXIX., 121.

[3] Loeb, J., Untersuchungen zur physiologischen Morphologie der Thiere, Würzburg, 1891–1892.

be reversibly prevented by placing them in sea-water concentrated by evaporation. This concentrated sea-water acted as an anesthetic. Loeb[1] also found that hypertonic solutions made by adding sodium chloride to sea-water prevented segmentation in the sea-urchin egg. The eggs were able to resume division on being transferred to normal sea-water, although the division process was somewhat altered.

I showed in 1920 as well as in 1915[2] that hypertonic solutions greatly increase the viscosity of sea-urchin egg cytoplasm. Hypertonic solutions are not the only anesthetics that act in this way. Dilute solutions of potassium cyanide have long been known as anesthetics for sea-urchin eggs. In anesthetic concentration, I have shown that cyanide solutions increase protoplasmic viscosity and render irreversible the normal gelation which occurs in the course of the mitotic process. Chloretone solutions act in a similar way.

Thus *there are two types af anesthesia in the sea-urchin egg.* In the one the viscosity of the cytoplasm is decreased, in the other it is increased. The two types of anesthesia differ also in the course of their action on the egg. When the viscosity is decreased sufficiently, the mitotic spindle is always prevented from forming. Even after the spindle has appeared exposure to ether or to cold causes the astral rays to disappear. On the other hand, anesthetics like hypertonic solutions and cyanide do not have such an effect. As is well known many hypertonic solutions produce asters and spindles. In dilute solutions of cyanide the mitotic process continues until the spindle is formed and then stops.

Of course in a way both types of anesthesia have one common effect upon the protoplasm. In both cases the protoplasmic viscosity is held fixed. Either the protoplasm is kept in a fluid state, or it is made to stay in a stiff condition. The end result is the same. The viscosity changes upon which mitosis depends can not occur, and the egg is anesthetized.

It is believed that not only in mitosis but in other processes as well, two types of anesthesia occur. This is borne out by a series

[1] Loeb, J., *J. Morph.*, 1892, VII., 253.

[2] Loc. cit.

of experiments now in progress with *Hydra*. Every student of zoölogy has watched this little animal contract on mechanical stimulation. I have been able to show that when *Hydra* is anesthetized, so that it no longer responds to mechanical stimulation, two types of anesthesia may be distinguished. In the one type, the *Hydra* tends to contract its tentacles and to remain contracted. This type of anesthesia is produced by ether and cold and in general by those substances which lower the viscosity of sea-urchin egg protoplasm. On the other hand, those substances which increase protoplasmic viscosity tend to anesthetize the *Hydra* in an expanded state.